Sub- and Supercritical Chiral Separation of Racemic Compounds on Columns with Stationary Phases Having Different Functional Groups

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Separation of the enantiomers of each of three different racemates, neutral *rac*-α-tetralol, acidic *rac*-2-phenylpropionic acid, and basic *rac*-1-phenylethylamine, using subcritical and supercritical fluid chromatography with two different chiral stationary phases, heptakis(2,3,6-*tri*-*O*-methyl)-β-cyclodextrin (Sumichiral OA-7500 column) and tris(3,5-dimethylphenylcarbamate) of amylose (Chiralpak AD-H column), was compared. The elution order of the enantiomers of the three racemates was determined, and the effects of the type of alcohol modifier, column oven temperature, mobile phase composition, flow rate, and pressure were examined. The most appropriate column oven temperature depended on both the type of alcohol modifier and the compound analyzed. Lower alcohol content improved the peak separation of both *rac*-α-tetralol on the Sumichiral OA-7500 column and *rac*-1-phenylethylamine on the Chiralpak AD-H column, while the same phenomenon was not observed with either *rac*-α-tetralol or *rac*-2-phenylpropionic acid on the Chiralpak AD-H column. Decreasing outlet pressure improved the peak separation obtained with *rac*-2-phenylpropionic acid, but had little effect on either *rac*-α-tetralol or *rac*-1-phenylethylamine.

Key words enantiomeric separation; supercritical fluid chromatography; chiral stationary phase; *rac*-α-tetralol; *rac*-2-phenylpropionic acid; *rac*-1-phenylethylamine

Chirality plays an important role in pharmaceutical research because of the different activities and toxicologic profiles of each enantiomer. Chemical synthesis of biologically active compounds with a stereogenic center generally yields a mixture of stereoisomers, such as enantiomers and diastereomers. Since the biological activity of each enantiomer differs, analytical methods to determine the enantiomeric purity of synthetic compounds are necessary, and chromatography to achieve peak separation on chiral stationary phases (CSPs) is currently the most widely used method.

Enantiomeric separation can be achieved using several different methods, including gas chromatography (GC),[1] high-performance liquid chromatography (HPLC),[2] and capillary electrophoresis.[3] And sub- and supercritical fluid chromatography (subFC and SFC)[4,5] has been demonstrated to be a good method for direct chiral separation. Carbon dioxide (CO_2) is widely used as an eluent for supercritical fluid because of its low cost, low toxicity, and easy handling. The advantages of subFC and SFC over HPLC include the higher diffusivity of test compounds and faster analysis times, which provide higher resolutions more rapidly. Furthermore, no derivatization of functional groups, which is sometimes required to increase volatility and thermal stability in GC, is necessary.

A wide range of CSPs has been used for subFC and SFC, including a brush-type (Pirkle-type) CSP,[6] macrocyclic antibiotics,[7] polysaccharide derivatives,[7—9] and native and derivatized cyclodextrins (CDs).[10—13] Commercially available columns representative of two major classes of CSPs were used in the present study: one based on a heptakis(2,3,6-*tri*-*O*-methyl)-β-CD derivative (Sumichiral OA-7500 column), and the other based on tris(3,5-dimethylphenylcarbamate) of amylose (Chiralpak AD-H column), as shown in Fig. 1. The results of enantiomeric separation on these two columns were compared.

The results of subFC and SFC on packed columns with CSPs based on native β-CD,[10,11] acetylated β-CD,[11] and (*R*)- or (*S*)-naphthylethylcarbamoylated β-CD[12,13] have been reported. The Sumichiral OA-7500 column bearing 2,3,6-*tri*-*O*-methyl-β-CD has been used for HPLC separation. In our previous study[14] we separated stereoisomers of menthol derivatives with HPLC using this column, and, to the best of our knowledge, this paper is the first report of enantiomeric separation using subFC and SFC on this column.

In this study, we attempted to separate the enantiomers of three racemic compounds (Fig. 1): a neutral compound, *rac*-α-tetralol (**1**); an acidic compound, *rac*-2-phenylpropionic acid (**2**); and a basic compound, *rac*-1-phenylethylamine (**3**). Their structures are simple, and they all contain a benzene ring that allows detection at ultraviolet wavelengths. α-Tetralol (**1**) is a chiral secondary alcohol used as a substrate[15] or starting material in enantioselective syntheses.[16,17] 2-Phenylpropionic acid (**2**) is an acidic compound that possesses a carboxyl group and is used to synthesize optically active compounds, for example, xanthine derivatives[18] and tetrapeptides.[19] 1-Phenylethylamine (**3**) is used in enantioselective syntheses of α-substituted primary amines[20] and as a chiral reagent in the preparation of enantiopure compounds.[21]

Investigating the enantiomeric separation of these compounds, which have fundamentally different structures and properties, was expected to be useful from both a fundamental perspective and in terms of general applications. In the previous reports[10,11] the peak selectivity obtained with SFC and subFC was higher than that obtained with HPLC, and in some cases the selectivity with subFC was superior to that with SFC.[6,12] We therefore attempted to perform enantiomeric separation using SFC and subFC. To identify the optimal conditions for enantiomeric separation, the effects of the type of alcohol modifier, column oven temperature, mobile phase composition, flow rate, and pressure were investigated.

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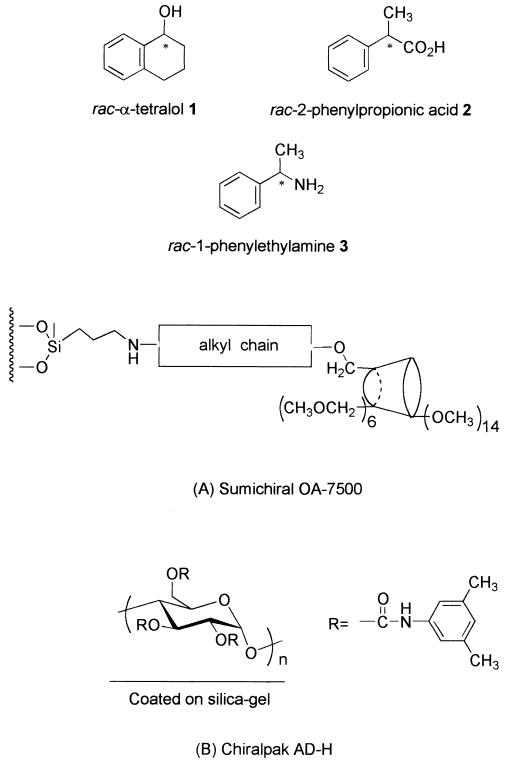


Fig. 1. Structures of the Compounds (**1**—**3**) Studied

The chiral stationary phases: (A) Sumichiral OA-7500; (B) Chiralpak AD-H.

Experimental

Chemicals Methanol (MeOH), ethanol (EtOH), and 2-propanol (2-PrOH), all of HPLC grade, were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The racemates *rac*-α-tetralol (**1**), *rac*-2-phenylpropionic acid (**2**), and *rac*-1-phenylethylamine (**3**) and optically active (−)-(*R*)-2-phenylpropionic acid and (+)-(*R*)-1-phenylethylamine, all of analytical grade, were also purchased from Wako Pure Chemical Industries, Ltd. Analytical grade (−)-(*R*)-α-tetralol was purchased from Sigma-Aldrich Japan (Tokyo, Japan). CO_2 was from Tomoe-shoukai (Tokyo, Japan).

The Sumichiral OA-7500 column (250×4.6-mm i.d.) based on heptakis(2,3,6-*tri*-*O*-methyl)-β-CD bound to a 5-μm silica-gel support and the Chiralpak AD-H column (250×4.6-mm i.d.) based on tris(3,5-dimethylphenylcarbamate) of amylose coated on a 5-μm silica-gel support were obtained from Sumika Chemical Analysis Service (Osaka, Japan) and from Daicel Chemical Industries, Ltd. (Tokyo, Japan), respectively.

SubFC and SFC Analyses SubFC and SFC were performed with an SFE/C-201 system (JASCO, Tokyo, Japan) equipped with an MD-910 multiwavelength detector and a CO-965 column oven. The pressure was controlled with a manual 880-81 back pressure regulator.

Compound **1** was dissolved in either MeOH or *n*-hexane at a concentration of 0.4 mg/ml, compound **2** was dissolved in MeOH at a concentration of 0.4 mg/ml, and compound **3** was dissolved in MeOH at a concentration of 0.3 mg/ml. A 10-μl sample of each solution was injected onto the column with a Rheodyne injector and monitored at a wavelength of 220 nm, which enabled detection of the benzene ring. The optically active compounds (−)-(*R*)-α-tetralol, (−)-(*R*)-2-phenylpropionic acid, and (+)-(*R*)-1-phenylethylamine were also separately injected under the same conditions as used for the racemates to confirm the elution order of the enantiomers.

Results and Discussion

Separation of *rac*-α-Tetralol (1) Peak separation of the enantiomers of compound **1** was achieved on both the Sumichiral OA-7500 and Chiralpak AD-H columns using alcohol-modified CO_2 as the eluent. Improved peak separations were achieved at 40 °C (SFC) with both the MeOH-modified CO_2 on the Sumichiral OA-7500 column (Fig. 2C) and the 2-PrOH-modified CO_2 on the Chiralpak AD-H column (Fig. 3A) in comparison with the separations at 25 °C (subFC).

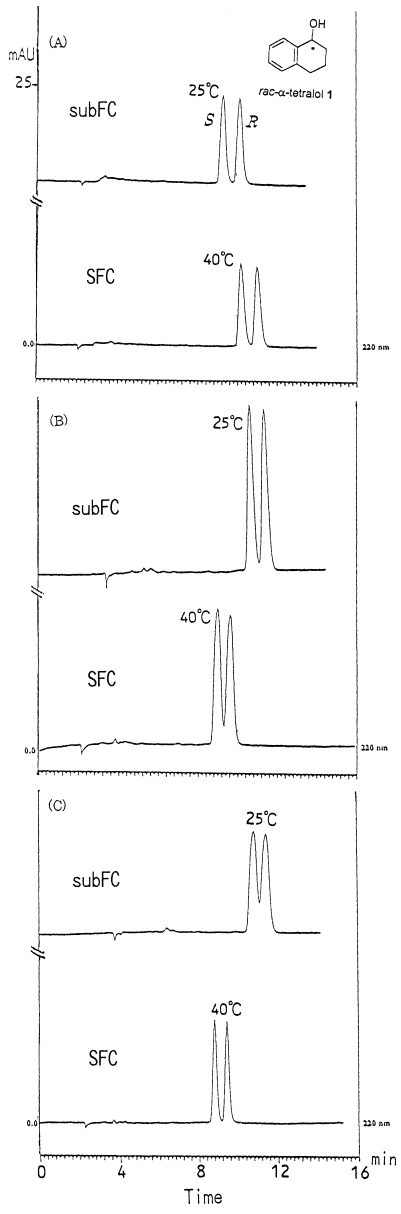


Fig. 2. Effect of Modifier Type and Column Oven Temperature on *rac*-α-Tetralol (**1**) Separation

Column: Sumichiral OA-7500. Conditions: flow rate, 2 ml/min; outlet pressure, 11.8 MPa; mobile phase, 2% (v/v) (A) 2-PrOH, (B) EtOH, (C) MeOH in carbon dioxide.

Sumichiral OA-7500 Column The chiral recognition process of CDs occurs in two steps: interaction with the secondary hydroxyl groups on the wider rim of CD and formation of an inclusion complex in the cavity of CD. Both the interaction between the solute and the rim of CD and inclusion of the solute molecule in the CD cavity are needed for a "tight fit" to achieve chiral discrimination governed by the steric fit between the CSP and the enantiomers.[10,22] The formation of reversible diastereomeric complexes between the compound analyzed and CD is simultaneously governed by different points of interaction with CD.

As shown in Fig. 2, peak separations of each enantiomer of *rac*-α-tetralol (**1**) were achieved in the alcohol (2-PrOH, EtOH, or MeOH)-modified CO_2 eluent investigated, and the (*S*)-enantiomer eluted first and was followed by the (*R*)-enan-

tiomer. To determine the optimal temperature of the column oven, analyses were performed at both 25 °C (subFC) and 40 °C (SFC).

This elution order suggests that the (*R*)-enantiomer interacts more strongly with CD than the (*S*)-enantiomer. The hydroxyl group of α-tetralol (**1**) is capable of interacting with 2- and 3-methylated hydroxyl groups on the wider rim of CD *via* hydrogen bonding and dipole–dipole interaction and forms a tight-fitting inclusion complex in the CD cavity, which leads to peak separation. The effect of the higher column oven temperature was greatest on MeOH-modified CO_2 (Fig. 2C). In the MeOH-modified CO_2 eluent the peak resolution (*Rs*) value of 1.65 at 40 °C was higher than the *Rs* value of 0.83 at 25 °C. Enantiomeric separation is affected by competition between the molecules analyzed and solvent used for specific adsorption sites on the CSP. In subFC (at 25 °C) the mobile phase became liquid, and the viscosity of the mobile phase increased in comparison with that of SFC (at 40 °C). Since MeOH is displaced more easily by the test compound at the recognition sites of CD at 40 °C than at 25 °C, the use of MeOH-modified CO_2 as the eluent at 40 °C may improve peak resolution.

In subFC or SFC, enantiomeric separation was achieved within a short analysis time. The small size of the CO_2 molecule suggests[10] that it could be displaced more easily by a compound analyzed at the CD cavity than by a hydrophobic solvent, *e.g.*, *n*-hexane, *etc.*, used in HPLC. Moreover, since only a small amount of alcohol modifier in CO_2 is needed for the mobile phase, there is no difficulty in disposing of an organic solvent.

Chiralpak AD-H Column As shown in Fig. 3, complete baseline separations of each enantiomer were achieved, with the (*S*)-enantiomer eluted first and then the (*R*)-enantiomer, in all three alcohol-modified CO_2 eluents examined.

The effects of column oven temperature were investigated by performing enantiomeric separation at 25 °C (subFC) and 40 °C (SFC). The effects of the higher temperature were greatest on 2-PrOH-modified CO_2 (Fig. 3A), and the higher temperature increased the α value from 1.19 to 1.29 and the *Rs* value from 3.26 to 5.75.

It is interesting to note that increasing the temperature resulted in longer retention times in the 2-PrOH-modified CO_2 eluent on both the Chiralpak AD-H (Fig. 3A) and Sumichiral OA-7500 (Fig. 2A) columns. At 40 °C (SFC), 2-PrOH is displaced more easily by the test compound at the recognition sites of the CSPs than at 25 °C (subFC), and longer inclusion time may be available for the test compound for enantiomeric recognition. This may be due to the longer aliphatic chain and higher Lewis basicity of 2-PrOH compared with EtOH and MeOH.

Separations were then performed using different compositions of the mobile phase, flow rates, and pressures. To ex-

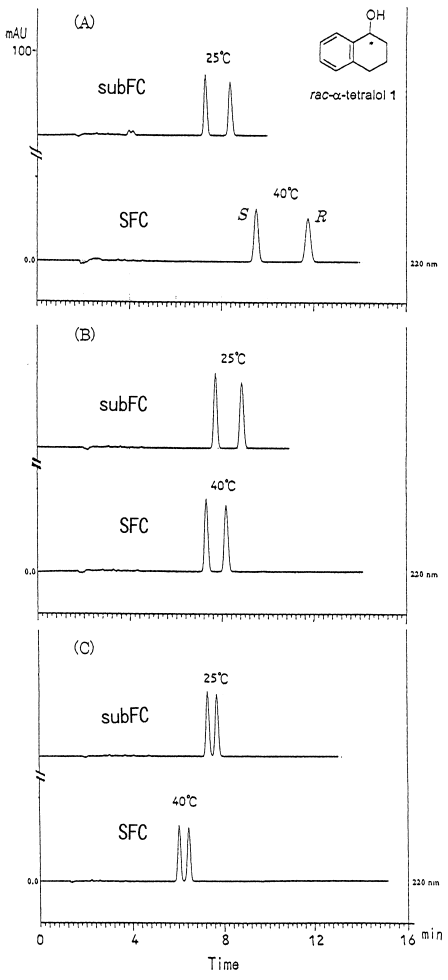


Fig. 3. Effect of Modifier Type and Column Oven Temperature on *rac*-α-Tetralol (**1**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 4 ml/min; outlet pressure, 13.7 MPa; mobile phase, 4% (v/v) (A) 2-PrOH, (B) EtOH, (C) MeOH in carbon dioxide.

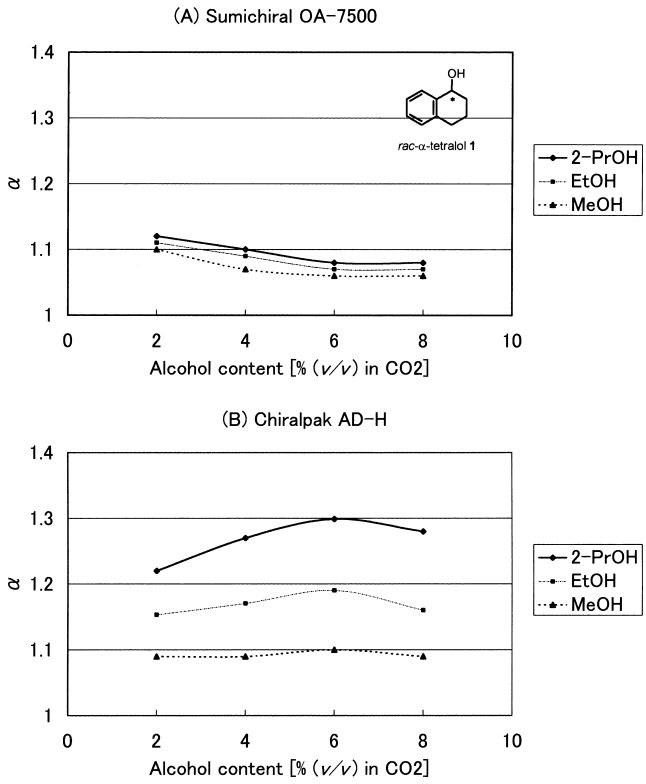


Fig. 4. Effect of Alcohol Modifier Content % (v/v) in Carbon Dioxide on *rac*-α-Tetralol (**1**) Separation

(A) Column: Sumichiral OA-7500. Conditions: flow rate, 2 ml/min; outlet pressure, 9.8 MPa; column oven temperature, 25 °C for 2-PrOH and EtOH (subFC), 40 °C for MeOH (SFC). (B) Column: Chiralpak AD-H. Conditions: flow rate, 4 ml/min; outlet pressure, 13.7 MPa; column oven temperature, 40 °C (SFC).

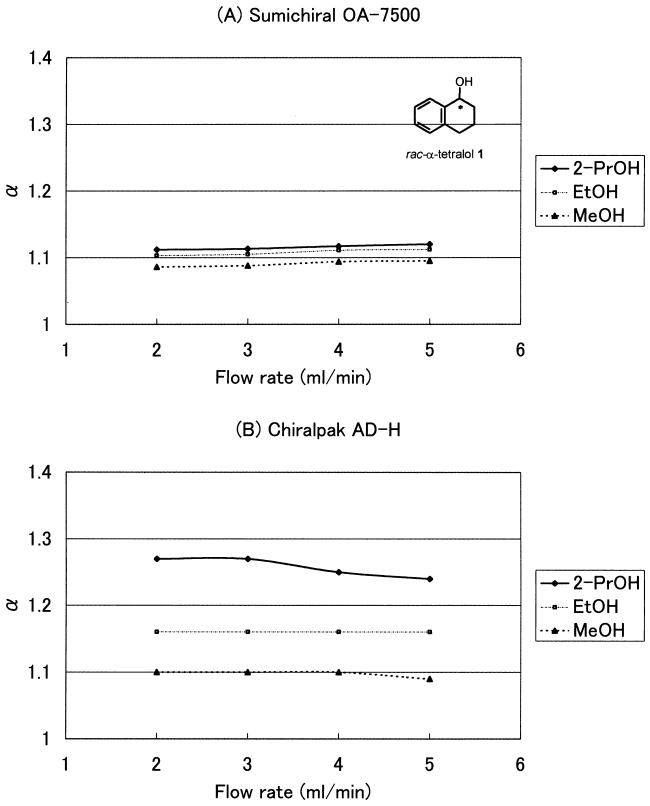


Fig. 5. Effect of Flow Rate of Mobile Phase on *rac*-α-Tetralol (**1**) Separation

(A) Column: Sumichiral OA-7500. Conditions: alcohol content in carbon dioxide, 2% (v/v); outlet pressure, 9.8 Mpa; column oven temperature, 25 °C for 2-PrOH and EtOH (subFC), 40 °C for MeOH (SFC). (B) Column: Chiralpak AD-H. Conditions: alcohol content in carbon dioxide, 4% (v/v); outlet pressure, 19.6 MPa; column oven temperature, 40 °C (SFC).

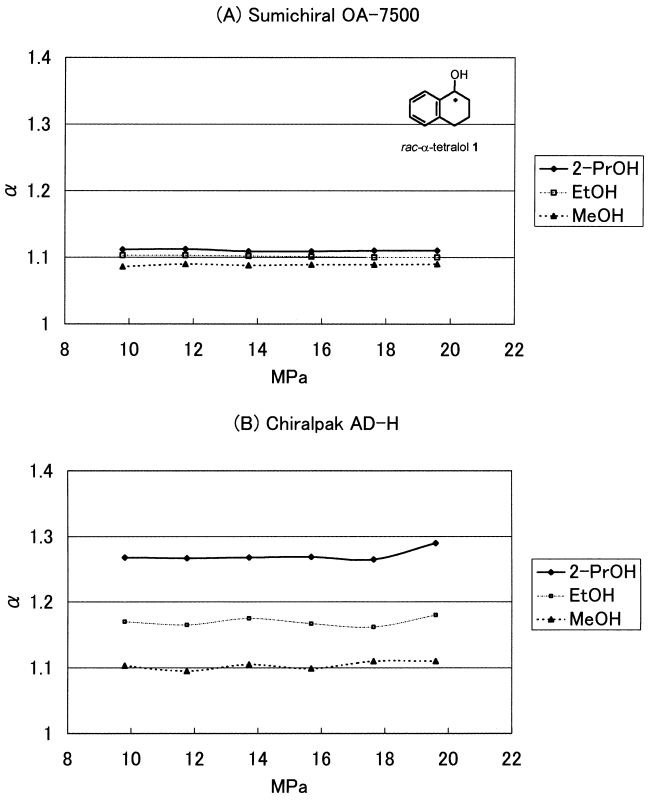


Fig. 6. Effect of Pressure on *rac*-α-Tetralol (**1**) Separation

(A) Column: Sumichiral OA-7500. Conditions: flow rate, 2 ml/min; alcohol modifier content in carbon dioxide, 2% (v/v); column oven temperature, 25 °C for 2-PrOH and EtOH (subFC), 40 °C for MeOH (SFC). (B) Column: Chiralpak AD-H. Conditions: flow rate, 3 ml/min; alcohol modifier content in carbon dioxide, 9% (v/v) for 2-PrOH, 4% (v/v) for EtOH, 2% (v/v) for MeOH; column oven temperature, 40 °C (SFC).

amine the effects of the dissolving solvent, both a MeOH solution and a *n*-hexane solution of compound **1** were prepared. However, the α values obtained differed only slightly.

Effects of Alcohol Content in CO_2 On the Sumichiral OA-7500 column, a decrease in the α value with increasing alcohol content was observed with every alcohol-modified CO_2 eluent investigated (Fig. 4A). The decrease in selectivity with increasing alcohol content appeared to be due to saturation of the chiral site by the polar alcohol modifier, which results in a reduction in the number of interactions between the compound analyzed and the CSPs. On the Chiralpak AD-H column, 6% (v/v) alcohol content in CO_2 improved peak selectivity with all alcohol-modified CO_2 eluents investigated (Fig. 4B).

Effects of Flow Rate Differences in the flow rate of the mobile phase had slight effects with all three eluents investigated. An increase in the α value with increasing flow rate was observed on the Sumichiral OA-7500 column, while the reverse was observed on the Chiralpak AD-H column (Fig. 5).

Effects of Pressure Differences in pressure had no significant effects on either column (Fig. 6).

2-PrOH always yielded higher selectivity and resolution than EtOH when used as a modifier on both the Sumichiral OA-7500 and Chiralpak AD-H columns, and even higher selectivity and resolution than MeOH. This may be related to steric hindrance at the α-position of the alcohol. Since straight-chain small alcohols attach more strongly to the chiral phase than branched-chain alcohols, it is more difficult for the compounds analyzed to displace them.

Separation of *rac*-2-Phenylpropionic Acid (2) Peak separation was achieved on the Chiralpak AD-H column, but not on the Sumichiral OA-7500 column, in the presence of any type of alcohol examined in CO_2. The transient diastereomeric complex formed through hydrogen bonding at the interaction site on CD is thought to be less stable than the complex formed in compound **1**. Carboxylic acid (**2**) releases protons more easily to form carboxylate ion, and prevents the tight-fitting interaction necessary for the discrimination process. Moreover, the inclusion of compound **2** may not be tight, and thus free rotation around the single bond inside the cavity of CD occurs and prevents chiral recognition.

Chiralpak AD-H Column Peak separation was achieved, with the (*R*)-enantiomer eluted first and then the (*S*)-enantiomer, with all the alcohol-modified CO_2 eluents examined. Enantiomeric separation was performed at 25 °C (subFC) and 40 °C (SFC) to investigate the effects of column oven temperature with each eluent. The chromatograms obtained are shown in Fig. 7. With the MeOH-modified CO_2 eluent, higher temperature increased the *Rs* value from 0.92 to 1.03.

Effects of Alcohol Content Separation with different alcohol contents in the mobile phase was performed. An alcohol content of 4% (v/v) in CO_2 resulted in improved peak selectivity with all alcohol-modified CO_2 eluents investigated,

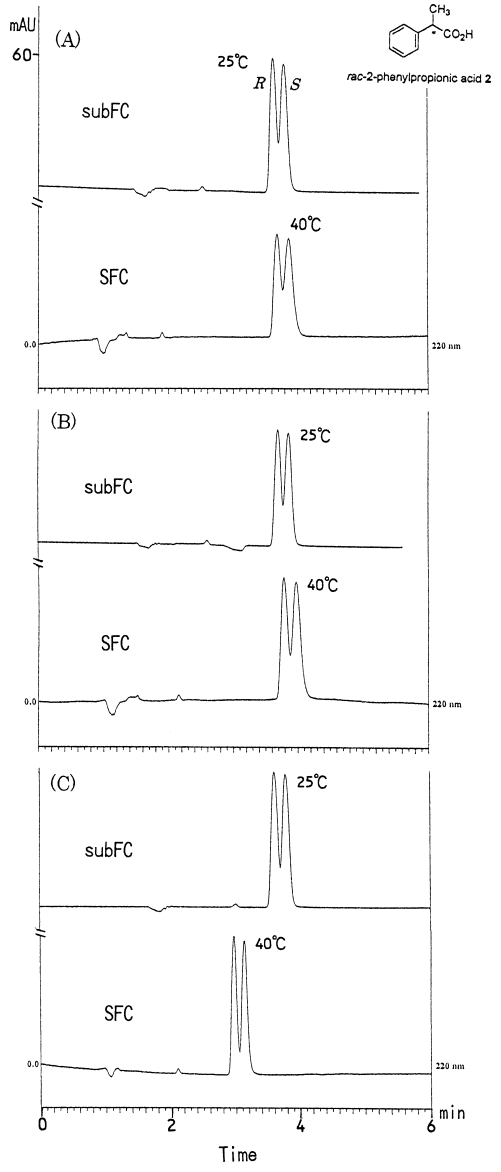


Fig. 7. Effect of Modifier Type and Column Oven Temperature on *rac*-2-Phenylpropionic Acid (**2**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 5 ml/min; outlet pressure, 7.9 MPa; mobile phase, 4% (v/v) (A) 2-PrOH, (B) EtOH, (C) MeOH in carbon dioxide.

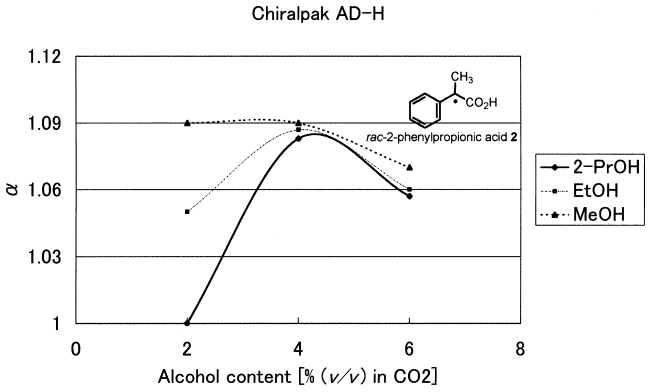


Fig. 8. Effect of Alcohol Modifier Content % (v/v) in Carbon Dioxide on *rac*-2-Phenylpropionic Acid (**2**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 5 ml/min; outlet pressure, 7.9 MPa; column oven temperature, 25 °C for 2-PrOH (subFC), 40 °C for EtOH and MeOH (SFC).

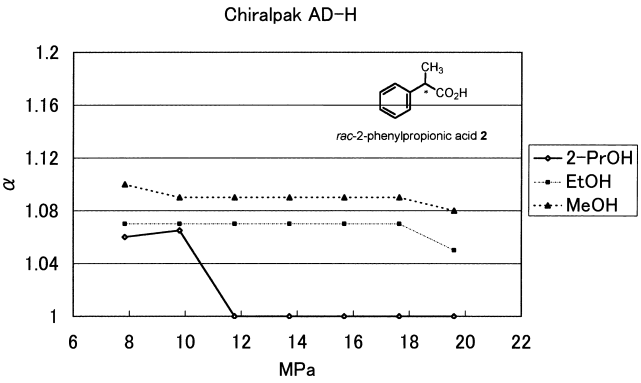


Fig. 9. Effect of Pressure on *rac*-2-Phenylpropionic Acid (**2**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 5 ml/min; alcohol modifier content in carbon dioxide, 4% (v/v); column oven temperature, 25 °C for 2-PrOH (subFC), 40 °C for EtOH and MeOH (SFC).

as shown in Fig. 8.

Effects of Pressure Separation at different pressures was then performed, as shown in Fig. 9. With the 2-PrOH-modified CO_2 eluent, peak separation did not occur at pressures of 11.8 MPa and greater. Lower pressure was more suitable for the separation of acidic *rac*-2-phenylpropionic acid (**2**), as observed previously in the enantiomeric separation of ibuprofen.[23]

Differences in flow rate had no significant effects on peak selectivity.

In the analysis of compound **2**, the α values decreased in the following order: MeOH-, EtOH-, and 2-PrOH-modified CO_2 eluent. For the separation of compound **2**, the small straight-chain alcohol MeOH is a more suitable modifier than larger alcohols, and the displacement at the binding sites of the CSP may occur more easily at 40 °C (SFC) than at 25 °C (subFC) in MeOH-modified CO_2. In a previous report,[24] enantiomeric separation of compound **2** with SFC was not observed when using a 5% (v/v) MeOH-modified CO_2 eluent on tris(4-methylbenzoate)cellulose derivative (Chiralcel OJ column).

Separation of *rac*-1-Phenylethylamine (3) SubFC and SFC with CO_2 as the eluent are known to be unsuitable for the separation of basic compounds because of the acidity of CO_2. Amino groups in basic compounds are prevented from interacting with the CSP by the formation of carbonate. Moreover, since alcohol modifiers are often not sufficiently strong to deactivate the surface properly,[25] basic or acidic additives are added to the mobile phase to improve the peak shape by masking the active sites of the support material. Diethylamine (DEA) was selected as an additive in this study to maintain the free amino function of the test compounds and mask the surface of the silica-gel support. Enantiomeric separation was thus achieved on the Chiralpak AD-H column. On the other hand, it was not achieved with any of the alcohol-modified CO_2 eluents examined on the Sumichiral OA-7500 column. Inclusion of the compound analyzed in the CD cavity may not be a sufficiently tight fit to recognize small differences in the reversible diastereomeric complexes, as previously assumed in regard to acidic compound **2**.

Chiralpak AD-H Column First, enantiomeric separa-

tion of compound **3** was attempted using MeOH-modified CO_2 as the eluent without any additive. As expected, no peak separation was observed, and DEA was added to the alcohol modifier to enable peak separation. To investigate the effects of the concentration of DEA in the alcohol modifier, separation was performed using MeOH containing 0.1%, 0.5%, or 1% DEA-modified CO_2 as the eluent. Since 0.1% DEA yielded the highest selectivity, subsequent experiments to determine the effects of temperature, mobile phase composition, pressure, and flow rate on peak selectivity were performed using alcohol containing 0.1% DEA-modified CO_2 as the eluent.

The chromatograms obtained with different types of alcohol containing 0.1% DEA-modified CO_2 eluents at 25 °C (subFC) are shown in Fig. 10. The (*R*)-enantiomer eluted first, followed by the (*S*)-enantiomer. In the 2-PrOH containing 0.1% DEA-modified CO_2 eluent, baseline separation was obtained at 25 °C, but no separation was observed at 40 °C (SFC). In both the EtOH containing 0.1% DEA- and MeOH containing 0.1% DEA-modified CO_2 eluents, the selectivity at 25 °C (subFC) was higher than that at 40 °C (SFC).

Effects of Modifier Content in CO_2 As shown in Fig. 11, with all modifiers examined, decreasing the content of alcohol containing 0.1% DEA increased selectivity. Decreasing the alcohol content increases the number of interactions for chiral discrimination, because the recognition is affected by competition between the test compound and solvent for specific adsorption sites on the CSP.

Differences in both flow rate and pressure had little effect on peak selectivity.

Thus, peak separation was achieved on the Chiralpak AD-H column with all types of alcohol containing 0.1% DEA-modified CO_2 examined in this study.

It is worth noting that both the *Rs* and α values of compound **3** tended to decrease in the order 2-PrOH-, EtOH-, and MeOH-modified CO_2, and the same phenomenon was observed with the neutral compound **1**. These results may be related to steric hindrance by the aliphatic chain of the alcohol, as mentioned above. The main adsorption site of the CSP is thought to be the polar amide functional group, which interacts with the solute *via* hydrogen-bonding, dipole–dipole interaction. The hydrogen bonding of small alcohols is stronger than that of large alcohols. Peak separation is influenced by competition between the test compound molecules and solvent for specific adsorption sites on the CSP. The greater difficulty with which test compounds displace small straight-chain alcohols may decrease the number of interactions for chiral discrimination, which results in low selectivity.

The optimal conditions obtained in this study and the separation parameters, *i.e.*, retention times (*t*), retention factors (*k*), α, and *Rs* are shown in Table 1. The separations of both *rac*-α-tetralol on the Sumichiral OA-7500 column and *rac*-1-phenylethylamine on the Chiralpak AD-H column were performed using subFC and the other separations were performed using SFC. The results obtained in this study will be applicable when similar compounds are analyzed using subFC or SFC.

Conclusions

Peak separation of the enantiomers of *rac*-α-tetralol was

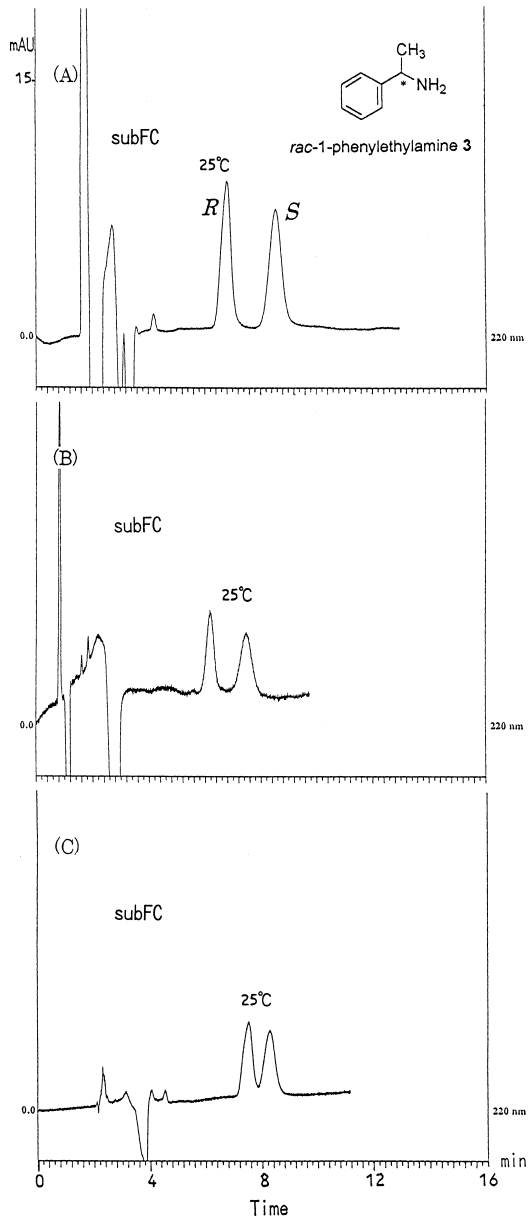


Fig. 10. Effect of Modifier Type on *rac*-1-Phenylethylamine (**3**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 5 ml/min; outlet pressure, 11.8 MPa; alcohol containing 0.1% DEA content in carbon dioxide, 4% (v/v) for (A) 2-PrOH and (B) EtOH, 2% (v/v) for (C) MeOH.

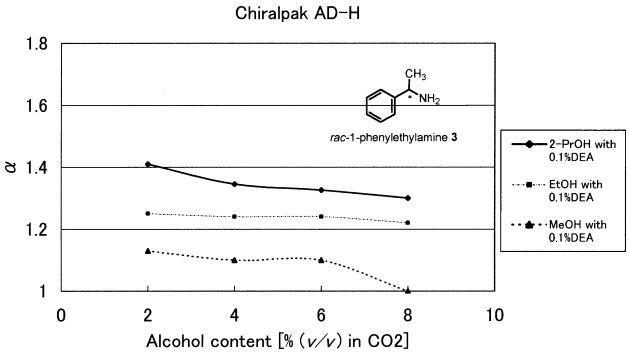
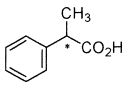
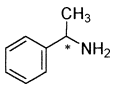


Fig. 11. Effect of Modifier Content % (v/v) in Carbon Dioxide on *rac*-1-Phenylethylamine (**3**) Separation

Column: Chiralpak AD-H. Conditions: flow rate, 5 ml/min; outlet pressure, 11.8 MPa; column oven temperature, 25 °C (subFC).

Table 1. The Optimal Conditions and Separation Parameters for the Enantiomeric Separations of the Racemic Compounds (**1**—**3**) Using subFC or SFC

Compound	Column	Alcohol % (v/v) in CO_2	Column oven temperature (°C)	Flow rate (ml/min)	Pressure (MPa)	t_1 (min)	t_2 (min)	k_1	k_2	α	*Rs*
rac-α-Tetralol 	Sumichiral OA-7500	2-PrOH 2%	25	5	9.8	3.8	4.2	2.8	3.2	1.1	2.2
	Chiralpak AD-H	2-PrOH 6%	40	4	13.7	5.9	7.4	4.9	6.4	1.3	7.5
rac-2-Phenyl-propionic acid 	Chiralpak AD-H	MeOH 4%	40	5	7.9	3	3.1	1.7	1.9	1.1	1
rac-1-Phenyl-ethylamine 	Chiralpak AD-H	2-PrOH with 0.1% DEA 4%	25	5	11.8	11	20	13.6	25.5	1.4	2.4

achieved using subFC and SFC on both the Sumichiral OA-7500 and Chiralpak AD-H columns, and 2-PrOH-modified CO_2 was appropriate as the eluent. On the Sumichiral OA-7500 column, the α and *Rs* values obtained with subFC were higher than those with SFC, while SFC was more appropriate for the Chiralpak AD-H column. The α values obtained on the Chiralpak AD-H column were higher than those on the Sumichiral OA7500 column. Enantiomeric separation of *rac*-2-phenylpropionic acid was achieved with MeOH-modified CO_2 eluent on the Chiralpak AD-H column with SFC. 2-PrOH containing 0.1% DEA-modified CO_2 eluent on the Chiralpak AD-H column resulted in improved peak separation of *rac*-1-phenylethylamine with subFC. The Sumichiral OA-7500 column did not separate enantiomers of either *rac*-2-phenylpropionic acid or *rac*-1-phenylethylamine under the conditions used in this study. The chromatographic results obtained in this study provide additional insight into the usefulness of subFC and SFC for chiral separation.

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